Plant Pathology (2009) **58**, 71–79 Doi: 10.1111/j.1365-3059.2008.01932.x

The interaction between *Meloidogyne arenaria* and *Cylindrocladium parasiticum* in runner peanut

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Cylindrocladium black rot (CBR), caused by *Cylindrocladium parasiticum*, and root-knot nematode, *Meloidogyne arenaria*, both infect and cause damage to the roots of peanut. Greenhouse and microplot experiments were conducted with the runner type peanut genotypes C724-19-15, C724-19-25 and Georgia-02C with different levels of resistance to nematode and CBR to better understand the interactions between the two pathogens. In the greenhouse, inoculation of 500–3000 eggs per plant of *M. arenaria* did not affect the level of root rot induced by 1·0 to 5·0 microsclerotia of *C. parasiticum* per g soil. In microplots, the root rot ratings from Georgia-02C and C724-19-25 were higher in plots infested with *M. arenaria* (0·4–2·0 eggs per cm^3 soil) and *C. parasiticum* than in plots with *C. parasiticum* alone; however, *M. arenaria* did not increase the root rot ratings on the nematode resistant C724-19-15. This was inconsistent with results in the greenhouse. Gall indices were not affected by *C. parasiticum* inoculations in the greenhouse or microplots. In both 2006 and 2007, a significant interaction between *C. parasiticum* inoculum densities and nematode level was observed on plant mortality. CBR inoculum greatly increased mortality on C724-19-25 and Georgia-02C, but not on C724-19-15, in the presence of *M. arenaria*. The mortality increase was more apparent at lower inoculum levels of both pathogens, but on the nematode-susceptible cultivars plant mortality was more with co-inoculations of the two pathogens than from either alone. Simultaneous inoculation with the two pathogens decreased yield of C724-19-25 and Georgia-02C as *C. parasiticum* inoculum levels increased, but even the largest inoculum of *M. arenaria* (2·0 eggs per cm^3 soil) did not decrease yield of C724-19-15.

Keywords: *Arachis hypogaea*, cylindrocladium black rot, parasite interactions, root-knot nematode

Introduction

Peanut (*Arachis hypogaea*) is a basic source of vegetable oil and proteins world wide, and is an important crop in the southeastern United States. The plants are unusual in that they flower above ground but the fruit (pods) develop underground. The root systems, as well as the pegs and pods, are susceptible to many soilborne pathogens. Two of the most important soilborne diseases of peanut in Georgia are root-knot nematode (*Meloidogyne arenaria* race 1) and cylindrocladium black rot (CBR), caused by the fungus *Cylindrocladium parasiticum*. *Meloidogyne arenaria* is prevalent in Alabama, Georgia, Florida and Texas, where as many as 40% of the peanut fields are infested with this pathogen (Ingram & Rodríguez-Kábana, 1980; Dickson, 1998; Koenning *et al*., 1999). Yield loss in heavily infested fields can be as much as 50%.

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Published online 11 August 2008

CBR was found first in 1965 in Georgia (Bell & Sobers, 1966), and it threatens peanut production throughout the southeastern United States (Harris & Beute, 1982). It is difficult to eradicate either of these parasites, and the number of fields infested with CBR and/or nematodes has apparently increased in recent years (T. B. Brenneman, University of Georgia, Tifton, GA, USA, personal communication). In Georgia alone, root-knot nematode and CBR cost peanut growers $11·4 and $4·3 million, respectively, in annual losses and costs of control from 2002–2006, according to University of Georgia extension service estimates (Martinez, 2006); they also reduced grades of peanut kernels.

Root-knot nematode and CBR are frequently found together in peanut fields (Diomande & Beute, 1981a; Culbreath *et al*., 1992), particularly those with poor crop rotations. There are several reports documenting a disease complex between root-knot nematodes and CBR on peanut (Diomande & Beute, 1981a,b; Diomande *et al*., 1981; Culbreath *et al*., 1992). *Meloidogyne hapla* increased CBR severity on both a CBR-resistant genotype, NC

3033, and a CBR-susceptible genotype, Florigiant (Diomande & Beute, 1981a,b). Culbreath *et al.* (1992) found that severity of CBR was increased in Florigiant by either *M. hapla* or *M. arenaria* with fungal inoculum densities of 0·05 and 0·5 microsclerotium per gram soil. Severity of black rot was not affected by either *M. hapla* or by *M. arenaria* on genotypes moderately resistant to CBR, such as NC 10C or NC Ac 18016 in the greenhouse. However, in the microplot experiments, root rot severity was enhanced by addition of *M. arenaria* or *M. hapla* on both CBR-susceptible and resistant genotypes. Earlier work showed that race 2 of *M. arenaria*, which is not a pathogen of peanut, also increased root rot caused by *C. parasiticum* on peanut (Diomande *et al.*, 1981). The genotypes used in all of these studies were virginia type peanut. However, runner type peanuts currently account for approximately 80% of the total US production, and interactions between *M. arenaria* and *C. parasiticum* on nematode-resistant or susceptible runner peanut genotypes have not been reported.

Significant progress has been made in breeding runner peanuts with resistance to *M. arenaria* and *C. parasiticum*. The first runner peanut cultivar offering partial resistance to *C. parasiticum* was released in 2002 (Branch, 2003). However, the resistance to *C. parasiticum* may be overcome when the cultivars are grown in *M. arenaria*-infested fields. Cultivars with high resistance to *M. arenaria* are also available (Simpson & Starrr, 2001; Simpson *et al.*, 2003; Holbrook *et al.*, 2008a). In order to effectively manage both the nematode and *C. parasiticum* in concomitantly infested fields, it is necessary to understand the interaction of *M. arenaria* and *C. parasiticum* on runner peanut with or without resistance to either pathogen.

The objective of this study was to assess the potential interactions between *M. arenaria* and *C. parasiticum* in determining the severity of the disease in runner peanut, particularly in CBR-resistant and Ma (*M. arenaria*)-resistant genotypes.

Materials and methods

Genotypes evaluated

Three runner peanut genotypes were used in this study: Georgia-02C is reported as moderately resistant to *C. parasiticum* and highly susceptible to *M. arenaria* (Branch, 2003), C724-19-15 and C724-19-25 are near isogenic breeding lines (Holbrook *et al.*, 2008b); C724-19-15 is highly resistant and was recently released as cv. Tifguard (Holbrook *et al.*, 2008a), while C724-19-25 is susceptible to *M. arenaria*.

Inoculum production

Fungal inoculum for greenhouse and microplot tests were produced on potato dextrose agar (PDA). Four isolates of *C. parasiticum* (CBR041, CBR0410, CBR0414 and CBR0418) obtained from infected peanut plants in southern Georgia in 2004 were used in all tests. To obtain microsclerotia, the isolates were grown on PDA for 6–7 weeks, after which the cultures were comminuted in a Waring Blendor for 2 min and passed through nested sieves with 250 μm and 150 μm openings (60 and 100 mesh, respectively). Microsclerotia in the 150-μm sieve were separated from mycelium fragments by passing a forceful stream of water through the sieve for 1 min. Microsclerotia were then rinsed into a 200-mL beaker containing about 100 mL of water. The concentration of the microsclerotia suspension was determined and adjusted with the aid of a microscope before use. Equal numbers of microsclerotia from each isolate were used in all tests.

Meloidogyne arenaria race 1, originating from a peanut field in Tifton, GA, was cultured alternately on tomato (*Solanum lycopersicum* cv. Rutgers) or eggplant (*S. melongena* cv. Blackbeauty) and peanut (cv. Georgia Green) to reduce potential contamination from *M. incognita* (a parasite of tomato and eggplant but not peanut). Eggs as inoculum for greenhouse experiments were extracted from tomato or eggplant roots by agitating in 0·6% NaOCl for 2–3 min (Hussey & Barker, 1973). The eggs were then collected and rinsed with tap water on nested 150- and 25-μm-pore sieves. Inocula of *M. arenaria* for microplot experiments was prepared as infested root tissue from cultures maintained on eggplant. After allowing 10 weeks for nematode reproduction, plants were harvested and the roots washed free of soil. The infected roots were cut into segments 2 to 3 cm long and chopped in a Waring Blendor for 2 min with water. Three samples (200 mL each) of this suspension of infected roots were collected to estimate the number of eggs and second stage juveniles (J2), and were shaken in 1·2% NaOCl for 5 min. Eggs and J2 were collected on a 25 μm (500 mesh) sieve and were counted with the aid of a stereo-microscope.

Greenhouse experiment

The three runner peanut genotypes (Georgia-02C, C724-19-15 and C724-19-25) were grown in all combinations of three *C. parasiticum*-inoculum densities (0, 1·0 and 5·0 microsclerotia per g soil) and three nematode levels (0, 500 and 3000 eggs per pot). Appropriate amount of inoculum suspensions were added to 3000 g premixed PRO-MIX 'BX' and Robin Hood top soil (1:1) in polyethylene bags. The infested soil was thoroughly mixed by shaking the soil in bags for 2 min. Each replication contained 27 experimental units. A split-plot treatment design was used with genotypes as main plots. Subplots of *C. parasiticum* density × *M. arenaria* level were randomized within ten replicate main plots. One seed was planted in each 10 × 10-cm square pot filled with 1000 g infested soil, and the pot was placed on a bench in the greenhouse at ~25°C. The bottom one-third of the pots were submerged in water for the duration of the experiment to provide a conducive environment for CBR. Plants were harvested after 8 weeks. Root rot ratings were visually estimated for CBR symptoms based on a 0 to 5 scale, where 0 = no symptoms; 1 = some root discoloration,

primarily on secondary roots; 2 = significant root browning and some necrosis, usually on secondary and tap root, with < 25% of roots affected; 3 = moderate root rot, 25–75% of roots affected; 4 = severe root rot, > 75% of roots affected; and 5 = dead plant. Gall indices were also assessed for nematode symptoms based on a 0 to 5 scale, where 0 = no galling; 1 = a few small galls; 2 ≤ 25% of root galled; 3 = 26–50%; 4 = 51–75%; and 5 > 75% of root galled (Dong *et al*., 2007). Whole plant fresh weight was measured, and the experiment was conducted three times during 2005–2006.

Microplot experiment

The microplot experiment was conducted at the Tifton Campus of the University of Georgia in 2006 and 2007. A total of 72 microplots were used in this experiment. Microplots were 170×140 cm^2 concrete, filled with Tifton loamy sand (fine-loamy, siliceous, thermic plinthic kandiudults) to a depth of 100 cm. Each plot was fumigated with metam sodium (Vapam) at 93·5 mL m^{-2} in 20 L of water 2 weeks prior to planting. Peanut was planted on 16 May 2006, and 22 May 2007. The experimental design was a split-plot, with *C. parasiticum* densities × nematode levels as main plots and genotypes as subplots over 2 years with eight replicates. Inoculum densities of *C. parasiticum* were 0, 0·5 and 5·0 microsclerotia per cm^3 soil (0, 3200 and 32 000 microsclerotia per plant), and nematode levels were 0, 0·4 and 2·0 eggs + J2 per cm^3 soil (0, 2500 and 12 700 eggs + J2 per plant), calculated for the top 20 cm of soil. The microplots were hand-planted with three 5-cm-deep furrows made by hoe (46·3 cm between rows and 170 cm long) in each main plot. Twenty-five seeds were planted in each row after the appropriate inoculum densities in 1000 mL water were applied in every furrow. Seeds were covered with soil, and herbicides (Sonalan 0·80 kg a.i. ha^{-1} + Dual Magnum 1·89 kg a.i. ha^{-1}) were applied for weed control within 3 days of planting. Chlorothalonil (1·2 kg a.i. ha^{-1}) was applied at 2- to 3-week intervals to control foliar fungal diseases. Plots were drench-irrigated as needed to maintain soil moisture at levels conducive for CBR development. The total plant numbers and the numbers of dead and wilted plants per subplot were counted at harvest (28 September, 2006 and 29 September, 2007). Plant mortality in each subplot was then calculated by dividing the number of dead and wilted plants by total plant numbers. Ten plants were randomly collected from each subplot for root rot rating and gall index based on the scales described previously. All peanuts were hand-picked and weighed after drying to approximately 10% moisture.

Data analysis

Data from three greenhouse trials were combined for analysis using Proc MIXED with ddfm = satterth option (a general Satterthwaite approximation for the denominator degrees of freedom) on the model statement (SAS v.9·1; SAS Institute, Cary, NC, USA), treating trials, replicates in trials, genotypes, nematode inoculum level, *C. parasiticum* densities and their interactions with trials as random effects. Microplot data from 2006 and 2007 were analysed separately by Proc MIXED of SAS, treating replicates and replicates × nematode levels × *C. parasiticum* densities as random effects, and treating all remaining sources as fixed effects. Any interaction effects that were not significant were removed and the reduced model was evaluated again. Main effects and interactions were considered significant when $P \leq 0{\cdot}05$. Fisher's least significant difference (LSD) values at $\alpha = 0{\cdot}05$ were computed using standard error and *t* values of adjusted degrees of freedom from the LSMEAN statement in Proc MIXED. Pearson correlation coefficients between different parameters were calculated by Proc CORR of SAS.

Results

Greenhouse experiment

The results from three greenhouse trials were combined for analysis. Significant difference ($P = 0{\cdot}0021$) was noted in root rot rating only for the main factor of *C. parasiticum* inoculum density, while peanut genotype, nematode level and all interactions were not significant ($P > 0{\cdot}05$). Root rot ratings generally increased in all three genotypes as the *C. parasiticum* inoculum densities increased (Table 1). The patterns of response to microsclerotia densities were similar at each nematode level.

The main factors peanut genotype, nematode level and the interaction of genotype × nematode level had significant effects on gall index ($P < 0{\cdot}05$). Moderate root and pod galling occurred on C724-19-25 and Georgia-02C at both 500 and 3000 nematode eggs per pot levels of nematode inoculum, and gall indices increased as the inoculum level increased (Fig. 1a). Gall indices were significantly lower in the resistant genotype C724-19-15 than in the other two genotypes. Inoculation with 500 to 3000 eggs per pot caused only a few galls on several plants

Table 1 Effect of inoculum densities of *Cylindrocladium parasiticum* (Cp) on root rot rating[a] of three peanut genotypes in the greenhouse

	Cp inoculum density (microsclerotia per cm^3 soil)		
Genotype	0	1	5
C724-19-15	1·0[b]	2·3	3·0
C724-19-25	1·4	2·5	2·9
Georgia-02C	1·1	1·9	2·5

[a]Root rot rating was on a 0 to 5 scale, where 0 = no symptoms; 1 = some root discoloration, primarily on secondary roots; 2 = significant root browning and some necrosis, usually on secondary and tap root, with < 25% of roots affected; 3 = moderate root rot, 25–75% of roots affected; 4 = severe root rot, > 75% of roots affected; and 5 = dead plant.

[b]Data were average across three trials (10 replicates/trial) and three inoculum levels of *Meloidogyne arenaria*; LSD(0·05) = 0·51 for comparison of *C. parasiticum* effects within and across genotypes.

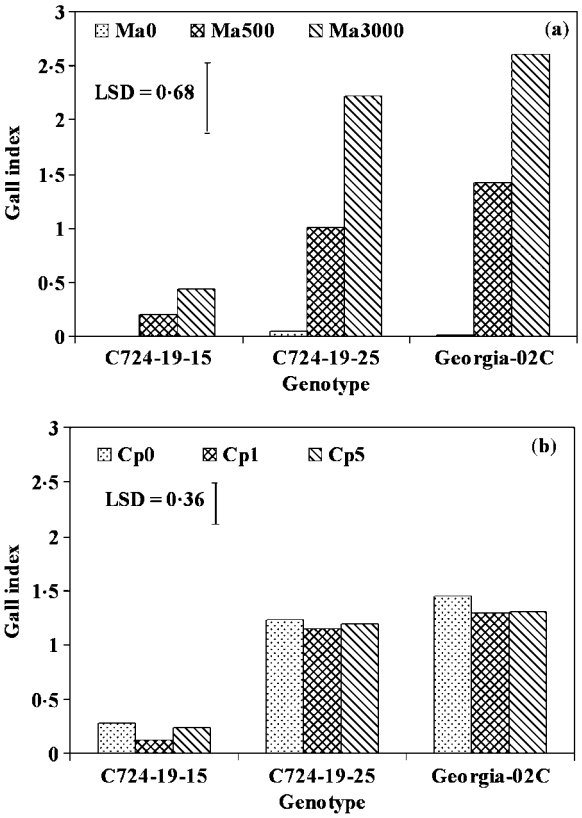

Figure 1 Gall indices for three peanut genotypes in the greenhouse in relation to inoculum density of *Meloidogyne arenaria* (a) and *Cylindrocladium parasiticum* (b). Bars represent the average across trials and *C. parasiticum* densities (a) or *M. arenaria* levels (b). Ma0, Ma500 and Ma3000 = 0, 500, and 3000 eggs per pot of *M. arenaria*; Cp0, Cp1 and Cp5 = 0, 1 and 5 microsclerotia per cm^3 soil of *C. parasiticum*. Gall index based on a 0 to 5 scale, where 0 = no galling; 1 = a few small galls; 2 ≤ 25% of root galled; 3 = 26–50%; 4 = 51–75%; and 5 > 75% of root galled.

of C724-19-15. Inoculum densities of *C. parasiticum* had no significant effects on the gall index in any of the three genotypes (Fig. 1b).

In the tests, only the main factor of *C. parasiticum* inoculum density affected ($P = 0{\cdot}023$) the whole plant weight. Significant decrease of the whole plant weight occurred in soil infested with 1 and 5 microsclerotia per g soil with C724-19-15 and C724-19-25 and only in soil inoculated with 5 microsclerotia per g with Georgia-02C (Fig. 2). The effects of the main factors of nematode level and genotype and the interactions were not significant ($P > 0{\cdot}05$). Correlation analysis showed a slight positive correlation between gall indices and fresh weights of plants ($r = 0{\cdot}16$, $P = 0{\cdot}005$), while root rot ratings were negatively correlated to fresh weights of plants ($r = -0{\cdot}15$, $P = 0{\cdot}008$).

Microplot experiment

In 2006, peanut genotype, *C. parasiticum* inoculum density ($P < 0{\cdot}0001$) and nematode level ($P < 0{\cdot}001$),

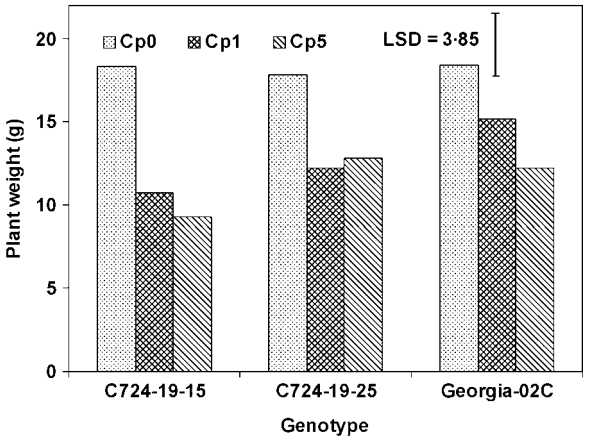

Figure 2 Effect of *Cylindrocladium parasiticum* inoculum density (microsclerotia per cm^3 soil) on fresh weight of plants of three peanut genotypes in the greenhouse. Bars represent the averages across three trials (10 replicates/trial) and levels of *Meloidogyne arenaria*. Inoculum densities of *C. parasiticum* were 0 (Cp0), 1 (Cp1) and 5 (Cp5) microsclerotia per g of soil. Georgia-02C is moderately resistant to CBR and highly susceptible to *M. arenaria*, and C724-19-15 and C724-19-25 are near isogenic breeding lines (C724-19-15 is highly resistant, while C724-19-25 is susceptible to *M. arenaria*).

as well as genotype × nematode level ($P < 0{\cdot}0001$) and genotype × *C. parasiticum* inoculum density ($P < 0{\cdot}05$) interactions had significant effects on root rot ratings, whereas other interactions did not ($P > 0{\cdot}05$). Root rot ratings on all three peanut genotypes generally increased as the *C. parasiticum* inoculum density increased (Fig. 3). In the absence of nematodes, there were no differences among root rot ratings on the three genotypes within the same *C. parasiticum* inoculum density. Root rot ratings on C724-19-25 and Georgia-02C plants grown in plots infested with both *C. parasiticum* and *M. arenaria* were higher than those infested with the fungus alone, especially at the higher level of *M. arenaria*. The nematode alone did not increase root rot on C724-19-15 and C724-19-25, but the high population did on Georgia-02C.

In 2007, all the main factors and interactions showed significant effects on root rot ratings ($P < 0{\cdot}05$). On all three peanut genotypes, root rot ratings generally increased as the *C. parasiticum* inoculum density increased from 0 to 5 microsclerotia per cm^3 soil, but often were not different ($P > 0{\cdot}05$) between 0·5 and 5 microsclerotia per cm^3 soil within the same nematode level (Fig. 3). However, at the highest rate of nematode inoculation, root rot rating on C724-19-15 with 0·5 microsclerotium of *C. parasiticum* was less than that with five microsclerotia, and in the absence of the nematode, root rot rating on C724-19-25 with five microsclerotia was greater than that with 0·5 microsclerotium. Similarly, root rot ratings on the three genotypes at the same *C. parasiticum* inoculum density were not different in the absence of nematodes. High populations of *M. arenaria* (2·0 eggs + J2 per cm^3 soil) alone caused root rot on all three genotypes, and even the low nematode level (0·4 eggs + J2 per cm^3 soil) significantly increased root rot

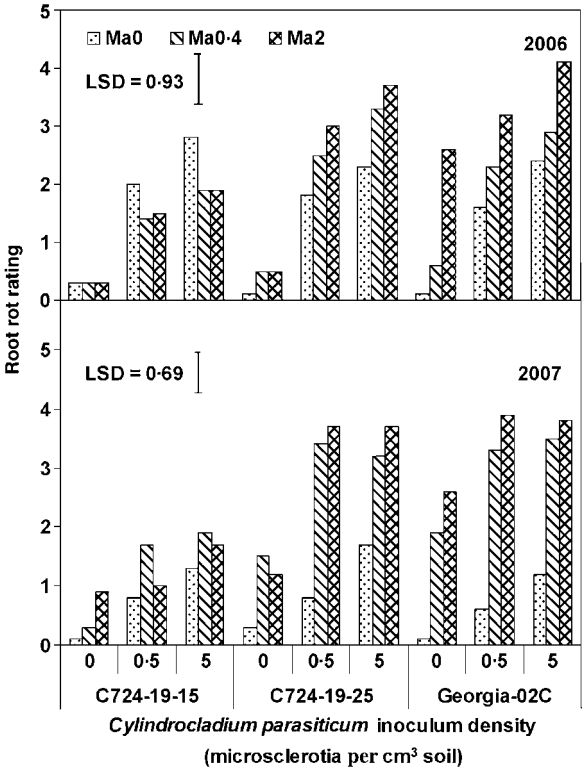

Figure 3 Root rot ratings for three peanut genotypes in microplots in 2006 and 2007, in relation to inoculum density of *Meloidogyne arenaria* and *Cylindrocladium parasiticum* (0 = healthy root system, 5 = completely rotted). Bars stand for the averages of eight replicates. LSD for comparison of *C. parasiticum* and *M. arenaria* effects within and across genotypes. Ma0, Ma0·4 and Ma2 represent the inoculum levels of *M. arenaria* of 0, 0·4 and 2 eggs + J2 per cm^3 soil (0, 2500 and 12 700 eggs + J2 per plant), respectively.

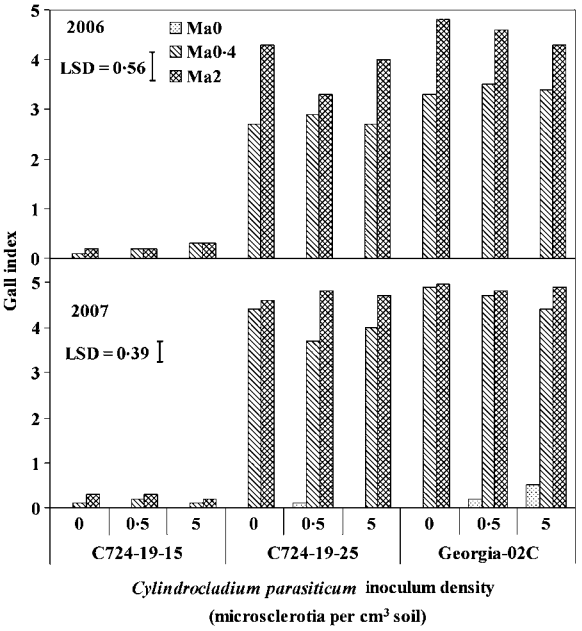

Figure 4 Gall indices for three peanut genotypes in microplots in 2006 and 2007, in relation to inoculum density of *Meloidogyne arenaria* and *Cylindrocladium parasiticum*. Gall index based on a 0 to 5 scale, where 0 = no galling; 1 = a few small galls; 2 ≤ 25% of root galled; 3 = 26–50%; 4 = 51–75%; and 5 > 75% of root galled. Bars stand for the averages of eight replicates. LSD for comparison of *C. parasiticum* and *M. arenaria* effects within and across genotypes. Ma0, Ma0·4 and Ma2 represent the inoculum levels of *M. arenaria* of 0, 0·4 and 2 eggs + J2 per cm^3 soil (0, 2500 and 12 700 eggs + J2 per plant), respectively.

ratings on C724-19-25 and Georgia-02C. Root rot ratings on plants grown in microplots infested with *C. parasiticum* and *M. arenaria* were higher than those infested with the fungus alone, with the exception of nematode-resistant genotype C724-19-15. An apparently synergistic interaction between *C. parasiticum* inoculum densities and nematode levels occurred on C724-19-25, but not on C724-19-15 and Georgia-02C (Fig. 3). On C724-19-25, root rot ratings caused by co-infestation at high levels of *M. arenaria* and *C. parasiticum* were significantly higher than the sum of those caused by the same levels of nematode and fungus alone (Fig. 3).

In both 2006 and 2007, the effects of genotype, nematode level and genotype × nematode level on gall index were significant ($P < 0{\cdot}0001$), whereas *C. parasiticum* inoculum density and the other two-factor or three-factor interactions were not ($P > 0{\cdot}05$). Only an occasional plant contained a low number of galls in plots without nematode inoculation, verifying that background populations of nematodes in the microplots was not an issue. In 2006, the high nematode level caused greater ($P \leq 0{\cdot}05$) root galling than the low nematode level on C724-19-25 and Georgia-02C in all but one comparison (Fig. 4). In 2007, even the low nematode level caused such severe galling on the nematode-susceptible genotypes that any additional effect of the high nematode level on galling was not apparent. By contrast, only a few galls were formed on the roots of C724-19-15 at the highest nematode inoculation level. Inoculum densities of *C. parasiticum* did not show significant effects on gall indices on any of the three peanut genotypes.

In both 2006 and 2007, dead and wilted plants were observed in all three peanut genotypes before harvesting, but the mortality was lower ($P \leq 0{\cdot}05$) in C724-19-15 than in Georgia-02C or C724-19-25 (Table 2). Genotype, *C. parasiticum* inoculum density, nematode level, genotype × nematode level, genotype × *C. parasiticum* inoculum density and nematode level × *C. parasiticum* inoculum density all affected ($P < 0{\cdot}01$) plant mortalities in both years. The three-factor interaction of genotype × nematode level × *C. parasiticum* inoculum density effect was also significant in 2007 ($P = 0{\cdot}0003$). In 2006 and 2007, *C. parasiticum* inoculum and *M. arenaria* alone or combined did not increase the mortality in the nematode-resistant genotype C724-19-15, and infestations with *C. parasiticum* inoculum alone frequently did not increase plant mortality in any of the genotypes. However, *C. parasiticum* inoculum strongly increased the mortalities

Table 2 Effect of *Meloidogyne arenaria* (Ma) and *Cylindrocladium parasiticum* (Cp) on mortality[a] in three peanut genotypes in microplots in 2006 and 2007

	Cp inoculum density (microsclerotia per cm^3 soil)								
	0			0·5			5		
Year	Ma level (eggs per cm^3 soil)								
Genotype	0	0·4	2	0	0·4	2	0	0·4	2
2006									
C724-19-15	2·7[b]	0	1·4	11·9	8·3	1·7	6·7	8·7	7·8
C724-19-25	0	1·3	19·2	6·3	19·7	33·9	8·5	34·9	54·6
Georgia-02C	1·3	6·2	45·7	2·9	23·0	38·1	1·8	42·6	67·0
2007									
C724-19-15	1·6	0·5	1·1	1·6	2·6	6·8	7·3	3·1	4·7
C724-19-25	0·5	4·7	8·9	0·5	54·7	80·7	1·6	38·0	59·4
Georgia-02C	0·5	25·5	48·4	1·1	45·8	57·3	6·8	64·1	67·2

[a]Plant mortality = (number of dead and wilted plants/total number of plants) × 100.
[b]Means of eight replicates. LSDs (0·05) were 14·0 and 9·4 for comparison of *C. parasiticum* and *M. arenaria* effects within and across genotypes in 2006 and 2007, respectively.

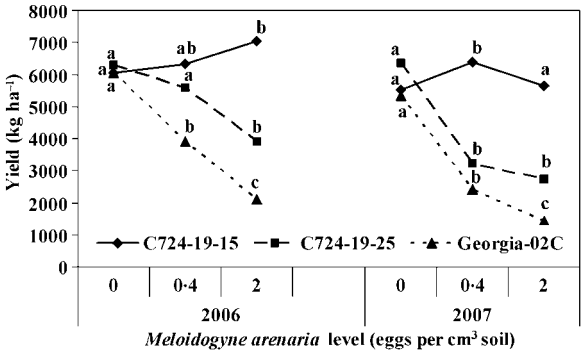

Figure 5 Effect of *Meloidogyne arenaria* on pod yield of three peanut genotypes in microplots in 2006 and 2007. Data points are averaged across inoculum levels of *C. parasiticum*. Means within a genotype followed by the same letter are not significantly different ($P > 0·05$).

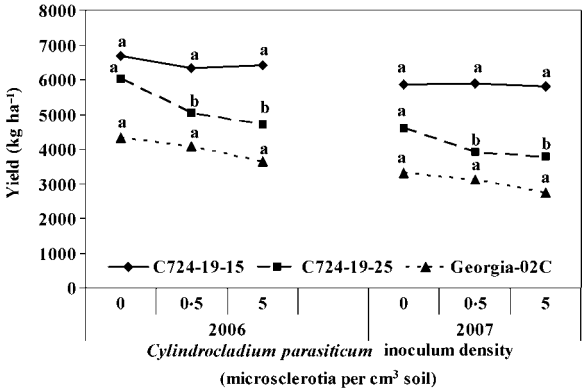

Figure 6 Effect of *Cylindrocladium parasiticum* on pod yield of three peanut genotypes in microplots in 2006 and 2007. Data points are averaged across inoculum levels of *M. arenaria*. Means within a genotype followed by the same letter are not significantly different ($P > 0·05$).

in C724-19-25 and Georgia-02C in the presence of *M. arenaria*. This was most evident at the lower inoculation levels. For example, with C724-19-25 in 2007, the low rate of *M. arenaria* and *C. parasiticum* inoculum alone caused only 4·7 and 0·5% mortality, respectively, whereas the combination of the two caused 54·7% mortality. Similar but less dramatic trends were observed in 2006, and with Georgia-02C in both years. The high level of nematode inoculum alone increased the mortality of C724-19-25 in 2006, while it increased the mortality of Georgia-02C in both 2006 and 2007. Of the main effects tested, nematode level, *C. parasiticum* inoculum density and plant genotype explained 14·4, 8·3 and 6·4%, and 19·5, 5·1 and 14·9% of the mortality in 2006 and 2007, respectively. This indicated that the environmental conditions were more conducive for nematode, but less conducive for CBR in 2007, which in fact was observed in other trials as well (unpublished data).

In 2006 and 2007, genotype, nematode level and *C. parasiticum* inoculum density, as well as the interaction of genotype × nematode level had significant ($P < 0·05$) effects on pod yield, but other interactions were not significant ($P > 0·05$). The three tested genotypes showed similar yields in the absence of *M. arenaria* (Fig. 5). The genotype C724-19-15 had much higher yield than the other two genotypes in the presence of the nematode (Figs 5 and 6). Yield reduction of C724-19-25 was less in 2006 (11·0%) than in 2007 (49·0%) as the nematode level increased from 0 to 0·4 eggs + J2/cm^3 soil, while it was greater in 2006 (43·4%) than in 2007 (19·2%) as the nematode level increased from 0·4 to 2 eggs + J2 per cm^3 soil. Similar trends were evident with Georgia-02C. Yield trends for the three genotypes in response to *C. parasiticum*

Table 3 Correlation coefficients between different parameters in microplot experiments in 2006 and 2007

Year Parameter	Gall index[a]	Mortality[b]	Yield
2006			
Root rot rating[c]	0·35*	0·69*	–0·34*
Gall index		0·33*	–0·20*
Mortality			–0·35*
2007			
Root rot rating	0·71*	0·82*	–0·71*
Gall index		0·72*	–0·81*
Mortality			–0·73*

[a]Gall index based on a scale of 0 to 5, where 0 = no galling; 1 = a few small galls; 2 ≤ 25% of root galled; 3 = 26–50%; 4 = 51–75%; and 5 > 75% of root galled.
[b]Plant mortality = (number of dead and wilted plants/total number of plants) × 100.
[c]Root rot rating based on a scale of 0 to 5, where 0 = no symptoms; 1 = some root discoloration, primarily on secondary roots; 2 = significant root browning and some necrosis, usually on secondary and tap root, with < 25% of roots affected; 3 = moderate root rot, 25–75% of roots affected; 4 = severe root rot, > 75% of roots affected; and 5 = dead plant.
*Correlations significant at $P \leq 0{\cdot}01$.

inoculation were similar in 2006 and 2007: yield of C724-19-25 decreased as the *C. parasiticum* inoculum density increased in the presence of *M. arenaria* (Fig. 6), but there were no differences in yield for genotypes C724-19-15 and Georgia-02C. This indicates that C724-19-15 may have a degree of tolerance to CBR in addition to its nematode resistance, especially since there were no interactions with nematode injury confounding the yield data.

In both 2006 and 2007, there were negative correlations between gall indices, root rot ratings and mortalities with pod yields ($P < 0{\cdot}01$) (Table 3). Positive correlations existed between each two of the three parameters, gall indices, root rot ratings and mortalities ($P < 0{\cdot}01$). The coefficient between gall indices and mortalities was higher in 2007 ($r = 0{\cdot}72$) than that in 2006 ($r = 0{\cdot}33$), suggesting that *M. arenaria* contributed more to the plant death in 2007.

Discussion

It was assumed that C724-19-15 and C724-19-25 had no resistance to CBR based on their pedigree. However, it was found that they had a similar level of moderate resistance to CBR as found in Georgia-02C. In addition, all three genotypes have good resistance to tomato spotted wilt virus (TSWV) (Branch, 2003; Holbrook *et al*., 2008b), which minimized the potential confounding effects of TSWV on root rot evaluation and pod yield (Culbreath *et al*., 1991).

Development of disease symptoms is dependent on the complex interrelationship among host, pathogen and prevailing environmental conditions. This is especially true for soilborne pathogens, because more opportunities exist for interactions with various other microorganisms occupying the same ecological niche. The important role of plant parasitic nematodes in the development of soilborne diseases has been demonstrated in many crops (Abdel-Momen & Starr, 1998; Rupe *et al*., 1999; Walker *et al*., 2000; Wheeler *et al*., 2000; De *et al*., 2001). Several fungus-nematode interaction studies have included peanut and *C. parasiticum* (Diomande & Beute, 1981a,b; Diomande *et al*., 1981; Culbreath *et al*., 1992). In nature, most infections of both *M. arenaria* and *C. parasiticum* occur near the root tips (Tomimatsu & Griffin, 1982; Hussey & Janssen, 2002). Therefore, it is conceivable that *M. arenaria* could influence the infection of *C. parasiticum* on peanut. However, the interactions between these two pathogens are not always detectable (Diomande & Beute 1981b; Diomande *et al*., 1981; Culbreath *et al*., 1992), because many factors beyond the pathogens may also affect the parameters which are used to determine the interactions.

Most researchers have determined the interactions between fungus and nematode based on symptoms (Diomande & Beute, 1981a,b; Diomande *et al*., 1981; Culbreath *et al*., 1992; Starr *et al*., 1996; Walker *et al*., 2000). The final populations of pathogens have also been used to evaluate interactions (Diomande & Beute, 1981b; Culbreath *et al*., 1992; Starr *et al*., 1996; Walker *et al*., 2000). In the current greenhouse experiment, root rot rating, gall index and plant weight were used to determine the individual and combined effects of *C. parasiticum* and *M. arenaria*. In the microplot experiments, root rot and galling were again evaluated as well as mortality and peanut yield, to examine the potential interactions between *C. parasiticum* and *M. arenaria*.

Root rot ratings have been used as an indicator of CBR severity on peanut. In the present study, root rot ratings were also significantly affected by *M. arenaria* in microplot experiments. Inoculating with *M. arenaria* alone increased the root rot ratings on the nematode-susceptible genotypes, Georgia-02C and C724-19-25. This was possibly caused by secondary infections of some other pathogens on *M. arenaria*-infected plants. In the current study, significant *C. parasiticum* inoculum density × nematode level interactions on plant mortality were observed in microplot experiments in both years. A synergistic interaction was also documented between *C. parasiticum* inoculum densities and nematode levels on root rot severity in C724-19-25 in the 2007 microplot experiment. However, the interactions of *C. parasiticum* inoculum density × nematode level on root rot severity were not significant for the greenhouse tests and the microplot experiment in 2006. No interactions were observed on yield in both years in the microplots. The high yield reductions on nematode-susceptible genotypes caused by *M. arenaria* alone may obscure the interactions of *C. parasiticum* inoculum density × nematode level. Similar inconsistent interactions have been observed in other studies (Diomande & Beute, 1981b; Diomande *et al*., 1981). Diomande & Beute (1981b) reported significant interactions between *M. hapla* or *Mesocriconema ornata*

and *C. parasiticum* in the field; however, there was no interaction between *M. ornata* and *C. parasiticum* on peanut genotype NC 3033 in greenhouse experiments. Diomande *et al.* (1981) found that root rot severity was increased in an additive manner when *M. arenaria* race 2 was combined with *C. parasiticum*.

Another factor that influenced the results was the inoculum densities used. More interactions between *M. arenaria* and *C. parasiticum* could have occurred if different nematode or fungal population densities had been tested. Population densities of both nematode and fungus affect development of the interaction. For example, in the disease complex of *M. incognita* and *Fusarium oxysporum* on tomato (Abawi & Barker, 1984) and peanut (Starr *et al.*, 1989), no interaction is typically observed if population densities of either pathogen are high or low.

Without the nematode and *C. parasiticum* present, the yields of the three genotypes were equivalent. The nematode-resistant genotype C724-19-15 was generally less severely diseased than the two nematode-susceptible genotypes in the microplots, and therefore had higher yield in the presence of *M. arenaria* with or without *C. parasiticum*. The fact that there is no yield depression associated with the nematode resistance makes it a logical choice for growers with even low levels of *M. arenaria*, particularly if CBR is a potential threat as well.

Several mechanisms have been proposed to explain the increased susceptibility of many nematode-infected plants to certain fungal pathogens (Back *et al.*, 2002). Wounding by the nematode (providing an entrance route for the fungus) was long considered important in increasing susceptibility to various fungi (Bergeson, 1972; Storey & Evans, 1987). Powell (1971), however, proposed that the increased capacity of certain *Meloidogyne* spp. to enhance fusarium wilt on tobacco when the nematode preceded the fungus by a few weeks is an indication of more elaborate mechanisms. Most artificial wounding in these types of tests does not realistically mimic nematode injury. The feeding sites of sedentary endoparasitic nematodes (giant cells or syncytia) are zones of high metabolic activity. These nutrient-rich cells could be the substrate for fungal colonization (McLean & Lawrence, 1993; Abdel-Momen & Starr, 1998). A 3–4 week nematode preinoculation has been found to be critical in investigations of some nematode-fungus disease complexes (Golden & van Gundy, 1975; Negron & Acosta, 1989). Taylor (1990) suggested this could be linked to syncytial development which will take 3–4 weeks to reach peak activity in a susceptible host. Although the mechanisms of resistance to *M. arenaria* in C724-19-15 have not been documented, based on its pedigree (Holbrook *et al.*, 2008b) it should be similar to cv. COAN, which restricts the formation of feeding sites but not initial penetration infective second stage juveniles (Bendezu & Starr, 2003). Therefore, the low root rot ratings in C724-19-15 cannot be completely explained by lack of wounds for *C. parasiticum* penetrating. Histological and physiological studies should be helpful to unveil the association between *M. arenaria* and *C. parasiticum* on peanut. This new cultivar (Tifguard = C724-19-15) will be an excellent tool for management of both root-knot nematode and CBR.

Acknowledgements

This research was supported in part by the Georgia Peanut Commission. The authors thank Patricia Hilton for her help in many aspects for this study. We also thank Jimmy Mixon, Lewis Mullis, Russell Griffin, Betty Tyler, Dannie Mauldin, Jason Golden and Amber Graham for their field and lab assistance, and Ben Mullinix for his excellent statistical assistance.

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